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IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY

14

INTRODUCTION		14.0.3
14.1	Fixation, Embedding, and Sectioning of Tissues, Embryos, and Single Cells Basic Protocol: Paraformaldehyde Fixation and Paraffin Wax Embedding of Tissues and Embryos	14.1.1 ,_ 14.1.1
	Alternate Protocol: Fixation of Suspended and Cultured Cells Support Protocol: Perfusion of Adult Mice	14.1.3 14.1.4
	Support Protocol: Sectioning Samples in Wax Blocks Reagents and Solutions Commentary	14.1.4 14.1.6 14.1.7
14.2	Cryosectioning Basic Protocol: Specimen Preparation and Sectioning	14.2.1 14.2.1
	Support Protocol: Fixation of Cryosections for In situ Hybridization Support Protocol: Tissue Fixation and Sucrose Infusion	14.2.5 14.2.6 14.2.6
14.3	In situ Hybridization to Cellular RNA Basic Protocol: Hybridization Using Paraffin Sections and Cells Alternate Protocol: Hybridization Using Cryosections Support Protocol: Synthesis of ³⁵ S-Labeled Riboprobes	14.3.1 14.3.1 14.3.5 14.3.7
	Support Protocol: Synthesis of ³⁵ S-Labeled Double-Stranded DNA Probes Reagents and Solutions Commentary	14.3.8 14.3.8 14.3.11
14.4	Detection of Hybridized Probe Basic Protocol: Film Autoradiography Basic Protocol: Emulsion Autoradiography Support Protocol: Preparation of Diluted Emulsion for Autoradiography	14.4.1 14.4.1 14.4.1 14.4.2
145	Commentary	14.4.3
14.5	In situ Hybridization Slides Basic Protocol: Giemsa Staining	14.5.1 14.5.1
	Alternate Protocol: Hematoxylin/Eosin Staining Alternate Protocol: Toluidine Blue Staining Alternate Protocol: Hoechst Staining	14.5.2 14.5.3 14.5.3
	Reagents and Solutions Commentary	14.5.3 14.5.4 14.5.5
14.6	6 Immunohistochemistry Basic Protocol: Immunofluorescent Labeling of Cells Grown as	14.6.1
	Monolayers Alternate Protocol: Immunofluorescent Labeling of Suspension Cells Basic Protocol: Immunofluorescent Labeling of Tissue Sections	14.6.1 14.6.2 14.6.3
	Alternate Protocol: Immunofluorescent Labeling of Tissue Sections Using Coplin Jars Alternate Protocol: Immunofluorescent Labeling Using	14.6.4
	Streptavidin-Biotin Conjugates Alternate Protocol: Immunogold Labeling of Tissue Sections	14.6.5 14.6.6
	Alternate Protocol: Immunoperoxidase Labeling of Tissue Sections Alternate Protocol: Immunofluorescent Double-Labeling of Tissue Sections Reagents and Solutions Commentary	14.6.6 14.6.7 14.6.7 14.6.8
		continued

SDS electrophoresis buffer, 5×

15.1 g Tris base

72.0 g glycine

5.0 g SDS

H₂O to 1000 ml

Dilute to 1× or 2× for working solution, as appropriate

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4°C until use (up to 1 month).

SED (standard enzyme diluent)

20 mM Tris-Cl, pH 7.5

500 μg/ml bovine serum albumin (Pentax Fraction V)

10 mM 2-mercaptoethanol

Store up to 1 month at 4°C

Sodium acetate, 3 M

Dissolve 408 g sodium acetate-3H₂O in 800 ml H₂O

Add H₂O to 1 liter

Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).

Solution B: 27.2 g sodium acetate (NaC₂H₃O₂·3H₂O)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g NaH₂PO₄·H₂O per liter (0.2 M).

Solution B: 53.65 g Na₂HPO₄·7H₂O per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H₂O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)

0.3 M Na₃citrate 2H₂O (88 g/liter)

Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris-Cl, pH 7.5

10 mM NaCl

1 mM EDTA, pH 8.0

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:

Working solution, pH ~8.5:

242 g Tris base

57.1 ml glacial acetic acid

40 mM Tris-acetate

37.2 g Na₂EDTA-2H₂O

2 mM Na₂EDTA-2H₂O

H₂O to 1 liter

TBE (Tris/borate/EDTA) electrophoresis buffer

10× stock solution, 1 liter:

108 g Tris base (890 mM)

55 g boric acid (890 mM)

40 ml 0.5 M EDTA, pH 8.0 (20 mM)

Appendix 2

A.2.5